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Perivascular Spaces and Their Role in Neuroinflammation

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Abstract

It is uncontested that perivascular spaces play critical roles in maintaining homeostasis and priming neuroinflammation. However, despite more than a century of intense research on perivascular spaces, many open questions remain about the anatomical compartment surrounding blood vessels within the central nervous system. The goal of this comprehensive review is to summarize the literature on perivascular spaces in human neuroinflammation and associated animal disease models. We describe the cell types taking part in the morphological and functional aspects of perivascular spaces and how those spaces can be visualized. Based on this, we propose a model of the cascade of events occurring during neuroinflammatory pathology. We also discuss current knowledge gaps and limitations of the available evidence. An improved understanding of perivascular spaces could advance our comprehension of the pathophysiology of neuroinflammation and open a new therapeutic window for neuroinflammatory diseases such as multiple sclerosis.

In Brief

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Author contributions

All the authors helped to design the study; BVI and SVO performed the literature review and data extraction; BVI wrote the manuscript; all the authors provided critical input to the final manuscript.

Compliance with Ethical Standards

Conflicts of interest

The authors declare no conflicts of interest related to the conduct of this study.

In this review, Ineichen and colleagues summarize the role of the perivascular space in neuroinflammation including involved cell types and how those spaces can be visualized. Based on this, they propose a model of the cascade of events during neuroinflammation.

Keywords

multiple sclerosis; neuroinflammation; perivascular spaces; paravascular spaces; Virchow-Robin spaces; review

1. A brief history of perivascular spaces

Physician-pathologist Rudolf Virchow was the first to publish a detailed description of a space surrounding certain brain vessels[1]. He termed it “disseziierende Ektasie” (dissecting ectasia) and drew a comparison with the pathology of an aortic aneurysm. Charles-Philippe Robin soon extended Virchow’s findings, postulating that these spaces were part of normal brain vessel anatomy[2]. Later, perivascular spaces became eponymous as Virchow-Robin spaces[3]. However, it must be noted that the term “Virchow-Robin space” now only relates to the prominent perivascular spaces surrounding larger arteries and veins, which can be seen macroscopically or on magnetic resonance imaging (MRI).

The potential functional significance of perivascular spaces was first suggested by Virchow’s pupil, Wilhelm His, who considered them the lymphatic analogue of the central nervous system (CNS)[4]. From then on, much research was devoted to perivascular spaces’ role in health and disease. This then somewhat abated until recent technological advances and the emergence of the glymphatic hypothesis once again fostered an interest in perivascular spaces.

This review provides an overview of the role of perivascular spaces in neuroinflammation. We first set the stage by discussing current concepts involving perivascular spaces and their associated controversies. Next, we summarize the literature on the role of perivascular spaces in neuroinflammation. Finally, we integrate the sum of the evidence on perivascular spaces, present a model of their role in neuroinflammation, and address unresolved gaps in knowledge.

2. Anatomy and visualization of perivascular spaces

2.1. Definition of perivascular spaces

The perivascular space is defined as the compartment surrounding the brain’s blood vessels — its arteries, arterioles, venules, and veins[5] (see Table 1 for definitions). There is a large body of historical and contemporary literature assessing the exact anatomical configuration of perivascular spaces, with considerable inconsistencies across studies, including in nomenclature. Indeed, there is an ongoing debate about whether these are true “spaces” or potential spaces that are normally filled with connective tissue. The most acknowledged current anatomical concept suggests that perivascular spaces are the compartments between the parenchymal basement membrane of the glia limitans (the outer boundary, formed by compacted astrocyte foot processes and an overlying parenchymal basement membrane) and

the endothelial basement membrane of the blood vessel (inner boundary)[6–8] (Figures 1A and 1B). Perivascular spaces are widely considered to be confined by the boundaries of the pia mater[1] (i.e., intracerebrally) and to follow the vascular tree down to the capillary level. At this point, the glial and endothelial basement membranes become closely juxtaposed and thus appearing as one structure[7] and obliterating the space[9–11].

2.2. Histopathology and electron microscopy

Because of their small dimensions, perivascular spaces have predominantly been studied using histological methods. Earlier studies employed less specific histological methods to visualize them[12]; more recent studies have used immunostaining. This has enabled a more specific identification of perivascular spaces, mostly by targeting antigens on the surface of astrocytic foot processes, e.g., aquaporins[13,14] and β-dystroglycan[15], but also certain laminin isoforms (of the parenchymal and endothelial basement membranes or of smooth muscle/mural cells) (Figure 2A)[7] and type IV collagen (a component of both basement membranes)[16]. Another solution has been the pan-laminin antibody that recognizes laminin α1, β1, and γ1 chains equally well, thereby labelling both acellular borders of the perivascular space[8] (Figure 2B). Electron microscopy has also been used to acquire ultrastructural insights into perivascular space anatomy and function[12,17,18] (Figure 2C).

2.3. Magnetic resonance imaging

In recent decades, literature on perivascular space histology and ultrastructure has been complemented by MRI studies[19]. Although MRI provides inferior resolution than tissue-based approaches for detecting perivascular spaces and thus only depicts the larger Virchow-Robin spaces, it does enable repeated noninvasive imaging of the entire brain. This is especially important considering potential changes in the configuration of perivascular spaces.

MRI identifies perivascular spaces as mostly linear structures with a signal intensity similar to cerebrospinal fluid (CSF)[20]. This has led to the identification and classification of enlarged perivascular spaces (EPVS) in specific brain regions into three types (Figures 2D–2F). Type 1 EPVS are situated in the basal ganglia along the lenticulostriate blood vessels (lentiform nucleus, external/internal capsule). In extensive cases, these are termed “état criblé” or “status cribrosus” and can also be seen on autopsy[21]. Type 2 EPVS are found in the centrum semiovale, and Type 3 in the midbrain (pontomesencephalic junction). Additionally, anterior temporal lobe perivascular spaces have been reported as a possibly distinct type[22]. Importantly, cortical perivascular spaces are difficult to detect using MRI, likely due to compaction of the glia limitans, pia mater, and vessel wall, as it has been shown in human and rodent cortices[23,24].

[1]Although this review focuses on perivascular spaces, for the sake of completeness, we also mention the term ‘paravascular’ spaces (from Ancient Greek: *para* – alongside). We suggest it be reserved for the spaces that are visualized alongside vessels in the subarachnoid or subpial space that have not been shown experimentally to completely surround the vessel. We use quotation marks around this term as it remains to be shown if this is a true anatomical compartment (see Table 1 and Figures 2J and 2K)

Recently, advanced MRI techniques using high static magnetic field strengths have improved the detection of even smaller perivascular spaces[25], enabling the classification of perivascular space location within the vascular tree, i.e., surrounding arteries or veins. By combining high-resolution MRI with susceptibility-based imaging to detect veins, one small study found that 80–90% of perivascular spaces adjoined arterial vessels[26], in line with findings from another small study employing high-resolution MRI[27].

Associations between enlarged perivascular spaces and neuroinflammatory diseases

Insights from MRI have confirmed previous knowledge from neuropathology by showing associations between EPVS and certain demographic parameters and/or diseases. Age[28] and hypertension[29] have been linked to higher numbers of arteries with enlarged perivascular spaces, and a recent large meta-analysis confirmed this[19]. Similar associations have been assessed in neuroinflammatory diseases: several studies have found greater numbers of EPVS in human inflammatory CNS diseases in general[30], in systemic lupus erythematosus (SLE)[31,32], and particularly in multiple sclerosis (MS)[33]. These findings in MS were recently corroborated by a systematic review and meta-analysis[34].

In MS, several studies have assessed associations between EPVS and clinical or imaging outcomes. Greater numbers of EPVS have been associated with worse cognitive performance[35], fatigue[36], the presence of gadolinium-enhancing lesions indicating opening of the blood–brain barrier (BBB)[37], lower brain volumes[25], and a lower percentage of central vein sign–positive (i.e., perivenular) lesions[38]. However, none of these findings has been replicated. Similarly, although one study found that higher numbers of basal ganglia EPVS were associated with lower expanded disability status scale (EDSS) scores, a composite measure of MS disability[39], these findings were not reproduced by other studies[25,35,37,40].

In SLE, greater numbers of centrum semiovale EPVS were associated with more disease activity[41]. Smaller studies and/or case reports have observed contrast enhancement around perivascular spaces in progressive multifocal leukoencephalopathy (PML)[42,43], (neuro)sarcoidosis[44], neuromyelitis optica spectrum disorder (NMOSD)[45], and primary CNS angiitis[46].

Perivascular spaces and cuffs in animal models

MRI has also been harnessed to study perivascular cuffs in experimental autoimmune encephalomyelitis (EAE), a commonly used neuroinflammatory animal model. Perivascular cuffs are leukocyte conglomerates within the perivascular spaces around postcapillary venules, formed before immune cells infiltrate the parenchyma. These perivascular cuffs are associated with opening of the BBB, as shown by the observation of iron-based contrast agent within perivascular cuffs at the level of postcapillary venules in murine EAE[47]. In marmoset EAE, such perivenular contrast enhancement can also precede the impending emergence of inflammatory plaques[48]. These early changes in vascular pathology corroborate the importance of the perivascular compartment also in experimental neuroinflammation.

3. Fluid circulation in perivascular spaces

3.1. Perivascular fluid dynamics

Following Goldmann's initial studies using vital dyes[49], the use of horseradish peroxidase and electron microscopy indicated that there was no barrier preventing the diffusion of macromolecules < 70 kDa between the subarachnoid space, perivascular spaces, and the brain's extracellular spaces[50,51]. The notion of perivascular fluid dynamics was confirmed by data from animal[52–55] and human studies[56–58]. This perivascular fluid drainage seems to decrease under certain conditions, e.g., with aging[59] or reduced vascular pulsations[60].

The presence of an intramural drainage pathway, i.e., within the vesse's muscular walls, further increases the complexity of what is referred to as perivascular fluid drainage[61,62] (reviewed in[10]).

Despite solid evidence of fluid dynamics within perivascular spaces, the potential route of entry for fluid into perivascular spaces is a controversial subject. The pia mater is the innermost layer of the meninges, coating veins and arteries in the subarachnoid space, and there is some evidence of a separation between the subarachnoid space and the subpial space[63]. The perivascular space containing interstitial fluid also seems to be separated from the subarachnoid space containing CSF[24]. This separation mainly involves cells and particulate material: in subarachnoid hemorrhage, erythrocytes do not enter the perivascular space. In contrast, there is no such barrier for inflammatory cells in meningitis[64]. There may be specialized pores (termed stomata) in the adventitial lining of leptomeningeal vessels, which could facilitate the exchange of cells or fluid between the subarachnoid and subpial/perivascular spaces[65,66]. Similar pores have also been observed in canine and human spinal cord pia mater, thus providing an additional fluid exchange route[67,68].

3.2. Molecular transport in the perivascular space

Tracer experiments have shown that molecules of up to 150 kDa can be transported within the intramural drainage pathway, whereas larger molecules, particularly cells or particulate material, track outside of arteries, adjacent to the glia limitans[62,69,70]. It has also been shown that tracers can be taken up by smooth muscle cells and perivascular macrophages along their passage[62]. It is possible that antigenic CNS material is delivered to perivascular macrophages in a similar fashion for presentation to trafficking lymphocytes[71].

Interestingly, this perivascular pathway might also be relevant for the distribution of gadolinium-based MRI contrast agents upon systemic application. Concerns about the use of such contrast agents were raised after gadolinium deposition was observed within deep gray matter structures[72–74], and the gadolinium may reach them via the perivascular fluid drainage system (reviewed in[75]).

Finally, it has been shown that β-amyloid is drained along this fluid drainage system but that its drainage becomes impaired with aging[76]. This impairment leaves insoluble β-amyloid deposits within basement membranes of the vessel walls, potentially further impeding efficient perivascular fluid exchange[77] and potentially giving rise to cerebral amyloid angiopathy and amyloid-associated imaging abnormalities[78].

3.3. The glymphatic hypothesis

CSF influx through perivascular spaces penetrating the CNS parenchyma was reported to be dependent on aquaporin-4 expressed on astrocyte end-feet[52,79]. Similarly, it has also been proposed that low-molecular-weight tracers (but also proteins such as HRP, albumin, and immunoglobulins) traverse the glia limitans to enter the parenchyma[23,52,53]. This process was originally termed convective tracer influx[53], but the concept was then extended and renamed the *glymphatic system*[52]. The glymphatic hypothesis suggests a periarterial influx of fluid followed by aquaporin-4-facilitated, convective, trans-parenchymal fluid drainage and, finally, perivenous efflux, thus representing a CNS-specific drainage circuit with a similar function to lymphatic vessels in other tissues[79,80].

Although this is an interesting concept, several lines of evidence do not support the glymphatic hypothesis, including those relating to the proposed role of aquaporin-4 (reviewed in[81] and[82]). For example, the high hydraulic resistance of the brain extracellular space might restrict convective flow in favor of diffusion[83,84]. Furthermore, pressure-dependent, aquaporin-4-mediated water entry into astrocytes might be prevented by the resulting oppositely-directed osmotic gradient[85]. In addition, flow from periarterial to perivenous spaces should depend on a hydrostatic pressure gradient, for which there is no evidence[86], and it has been suggested that net parenchymal flow could be explained by diffusion alone[87]. Finally, the existence of perivascular fluid influx and efflux remains controversial as animal studies utilizing injected tracers have reported conflicting findings on flow direction[23,52,88] and changes in tracer distribution at the time of death, limiting the value of post-mortem analysis[55]. Indeed, key aspects of the original studies[52] have proved difficult to reproduce, such as the reduced perivascular fluid flow in aquaporin-4-null mice[87]. Taken together, although there is strong evidence of fluid dynamics along and through perivascular spaces, more research is needed to elucidate the nature and direction of the parenchymal fluid exchange pathways proposed by the glymphatic hypothesis.

4. Immune cells and other cells in perivascular spaces

In addition to their role in fluid dynamics, immunocytochemical studies have confirmed the presence of scattered CD45-expressing cells in perivascular spaces (reviewed in[89] and[90]) (Figure 1C). The presence of myeloid cells with macrophage-like properties or dendritic cells in the perivascular space has two potential implications: first, it provides an opportunity for foreign antigens to be taken up and processed by these resident antigen presenting cells (APCs)[91–93]; second, it allows for interactions between antigen-loaded macrophages/dendritic cells and lymphocytes from adjacent blood vessels or CSF[94].

The second notion requires that lymphocytes enter perivascular spaces. During physiological conditions, such lymphocytes may be recruited from the CSF and seem to actively surveil the perivascular spaces (reviewed in[95]). Lymphocytes can also enter the perivascular spaces from the bloodstream across the endothelial barrier[96]. Many researchers have contributed to analyzing such CNS barriers, including Stern[97,98], Lewandowsky[99], Goldmann[49], and Reese and Karnovsky[100] (reviewed in[101]). However, Stern and Gautier were the first to use the term blood–brain barrier (BBB), in 1918, in their study of how a wide range of molecules from the blood penetrated into CSF[101,102].

Lymphocytes such as activated or effector/memory T cells can enter the meninges and perivascular spaces independently of their antigen-specificity and are further activated when they recognize their cognate antigen present on local border-associated macrophages or dendritic cells in the subarachnoid space. This was shown in murine spinal cords using two-photon intravital microscopy[71,103–105]. Upon antigen-specific activation in subarachnoid or perivascular spaces, T cells gain the ability to enter the CNS parenchyma via migration across the glia limitans, potentially causing inflammatory tissue damage that results in clinical symptoms[8,106] (reviewed in[107]). This has been shown in EAE models in which T cells recognized an antigen present within the CNS parenchyma (reviewed in[108]).

Importantly, during BBB disturbance, immune cell recruitment occurs at postcapillary venules[61,109]. This is in contrast to the diffusion of soluble molecules across the cerebral endothelium which is governed by different mechanisms and which is mostly controlled at the capillary level[110].

5. Functional aspects of perivascular spaces in neuroinflammation

The opening of the BBB is a key process of neuroinflammation. Although traditionally considered a single entity, the BBB comprises two anatomical layers, the endothelial cell/endothelial basement membrane layer and the glia limitans (formed by compacted astrocyte foot processes and an overlying parenchymal basement membrane), which are separated by a distinct perivascular space except at the capillary level (Table 1 and Figure 1). Interestingly, data suggest that penetration of immune cells through the endothelial layer and their process of parenchymal invasion across the glia limitans are distinct processes, independent of one another[111]. Thus, for leukocytes, perivascular spaces are not merely another compartment to cross to access the CNS parenchyma; rather, they provide the critical components needed to initiate neuroinflammation and CNS immune surveillance.

(1) Endothelial barrier

Immune cell migration through the endothelium is a tightly controlled process involving various cell types and key molecules, reviewed in[112–114].

Chemokines and their receptors—Several chemokine receptors have been shown to govern immune cell recruitment to perivascular spaces through the endothelium during neuroinflammation, among them chemokine receptors 7 and 8 (CCR7 and CCR8), which are upregulated during early EAE relapses and expressed on inflammatory cells in perivascular spaces[115].

Chemokine ligands such as CCL2, secreted by microglia[116] and astrocytes[117], also play a role. In murine EAE, the lack of astroglial CCL2 reduced the capacity of CD4+ T cells to transit from perivascular spaces into the spinal cord parenchyma[117]. Another series of studies in a transgenic mouse model overexpressing CCL2 found that inflammatory cells were confined to the perivascular space[118]. However, additionally challenging these mice using pertussis toxin induced cellular infiltration and fluid leakage to the parenchyma[119,120]. Together, these findings emphasize that CCL2 overexpression is insufficient to induce active CNS inflammation; additional cues are required such as other chemokine ligands, e.g.,

CXCL10. In murine EAE, the deletion of astroglial CXCL10 reduced the influx of CD4+ T cells into spinal cord perivascular spaces, but macrophage accumulation was unaffected[121].

Endothelial cells and molecules—It is not surprising that aside from leukocytes and glial cells, endothelial cells also regulate the transmigration of immune cells into perivascular spaces. When membrane proteins of inflammatory cells, such as CD99L2[122], that are typically expressed on inflammatory cells, are also expressed on endothelial cells, they seem to be critical molecules for enabling transmigration through the endothelial barrier into perivascular spaces.

Additional endothelial molecules important for immune cells to cross the endothelial barrier are claudin-5 and TNF receptor 1 (TNFR1). In EAE, endothelial cells are able to transmit molecules like claudin-5 to circulating leukocytes, thereby enabling their passage through the endothelial cell layer[123]. Finally, TNFR1, which is expressed on endothelial cells and a variety of glial cells, is important for other peripheral inflammatory cells, such as macrophages, to reach the perivascular space, also resulting in lower production of proinflammatory chemokines[116].

(2) Sojourn in the perivascular space

Data from rodent[8,124] and marmoset EAE[48] models suggest that inflammatory cells can be present within perivascular cuffs for up to several weeks before inflammatory parenchymal demyelination. Yet only a few studies have addressed the processes occurring during this sojourn.

Observations from *in vivo* microscopy of rodent EAE lesions showed that CD4+ T cells seem to be compartmentalized within perivascular spaces unless they recognize their cognate antigen on perivascular APCs[71]. A follow-up study in a similar model and employing *in vivo* microscopy of spinal meningeal vessels found that CD4+ T cells were activated by macrophages and potentially other APCs[125]. This would be in line with observations from murine viral encephalomyelitis models where CD8+ T cells were directly recruited to the parenchyma, whereas CD4+ T cells accumulated in perivascular spaces before transiting to the parenchyma[126]. A similar mechanism has also been proposed for myelin oligodendrocyte glycoprotein antibody-associated disease (MOGAD), in which MOG-loaded macrophages were observed in perivascular spaces[127].

But macrophages may also serve additional purposes besides antigen presenting properties: they can secrete proinflammatory cytokines such as interleukin-23, which has been found in active and chronic MS lesions and which could further stimulate autoreactive T cells locally[128]. Furthermore, fibrin deposition associated with astrocytic swelling has been reported in perivascular spaces in MS, which might also promote the parenchymal infiltration of immune cells[129,130].

(3) Parenchymal border

Similar to the endothelial barrier, migration through the parenchymal border is a highly controlled process governed by various key molecules.

Matrix metalloproteinases—MMPs also seem critical to immune cell trafficking from the perivascular space to the CNS parenchyma. Gelatinase activity in early neuroinflammatory lesions has initially been shown in murine EAE[15]. This notion is further supported by the observation that there is significant MMP expression in early, active MS lesions[131]. Also, molecular imaging of gelatinase activity has been used as early marker of leukocyte infiltration in MS[132]. Indeed, in EAE, both MMP2 and MMP9 (also known as gelatinases A and B) aid leukocyte transmigration by modulating chemokine activity and thus generating a chemotactic gradient that attracts T cells from the perivascular space to the parenchyma[133]. Consistent with these observations, MMP-antagonizing molecules are also involved in parenchymal infiltration: upregulation of tissue inhibitor of metalloproteinase 1 (TIMP-1) by astrocytes inhibits parenchymal leukocyte infiltration from perivascular spaces[134]. Similarly, depletion of peripheral macrophages, which are potential secretors of MMPs, resulted in higher numbers of T cells being confined to perivascular spaces in EAE[111,135].

MMP expression is governed by the MMP-inducer EMMPRIN (CD147), which is expressed on endothelial cells, leukocytes, and microglia. Its upregulation was associated with more severe EAE[136,137], and its pharmacological inhibition dampened EAE severity[138]. Intriguingly, EMMPRIN also seems to regulate monocarboxylate transporter 4 (MCT-4), a potent secretor of lactate[139]. Indeed, it has been shown that macrophages in the perivascular cuff are highly glycolytic, expressing both lactate dehydrogenase A (converting pyruvate to lactate) and MCT-4. Knockdown of these molecules resulted in decreased lactate secretion and reduced transmigration from perivascular spaces to the parenchyma.

Among other substrates, dystroglycan, which anchors astrocyte end-feet to the parenchymal basement membrane, is a major substrate for MMPs[15]. It has also been shown that the platelet endothelial cell adhesion molecule (PECAM-1) might complement MMPs during leukocyte transmigration[140].

Chemokines and their receptors—Chemokine receptors and their ligands are also involved in parenchymal infiltration of immune cells. Just as it facilitates transendothelial transmigration, CCL2 regulates trafficking of inflammatory cells to the parenchyma from perivascular spaces. In EAE, dendritic cells and T cells migrate across the glia limitans into the parenchyma in a CCL2-dependent manner[141]. In a murine viral neuroinflammatory model, CCL2 ablation resulted in CD4+ and CD8+ T cells being retained in perivascular spaces, causing a delayed control of viral neuroinflammation[142].

The chemokine ligand CXCL12 and its receptor CXCR4 also play an important role in migration of inflammatory cells to the CNS parenchyma[143,144]. It has been shown in EAE that T cells which express CXCR4 are confined to the CXCL12-enriched perivascular space[143]. Upon inflammation, CXCR7 is upregulated on the endothelium resulting in uptake and degradation of CXCL12[145]. These reduced CXCL12 concentrations in the perivascular space promote a pro-inflammatory environment by allowing T cells to migrate towards the CNS parenchyma in a chemokine-dependent manner[133]. Of note, MMP2 and MMP9 act to inactivate CXCL12 in the perivascular cuff thereby enabling immune cells to respond to other chemokines expressed outside of the perivascular cuff[132,146]. These gelatinases

also selectively cleave several CCL chemokines, including CCL2, antagonizing CCR receptors[147]. Hence, MMP activity at the parenchymal border acts to fine tune chemotactic signals across the parenchymal border[148].

Additionally involved molecules—Another relevant process is TNFR1-dependent VCAM expression on astrocytes, which is involved in T cell trafficking from perivascular spaces to the parenchyma[106]. In line with this observation, TNF-deficient mice with EAE show inflammatory cells entrapped within their perivascular spaces[149].

Several additional molecules have been suggested as promoters of immune cell trafficking from perivascular spaces to the parenchyma in neuroinflammatory animal models, including pericytic aminopeptidase N[150], complement factors B and C3[151], astrocytic junctional adhesion molecule-A[152], and certain chondroitin sulfate proteoglycans[153]. These proteoglycans[153], urokinase plasminogen activator, urokinase receptor, and plasminogen activator inhibitor-1 were all upregulated in acute MS lesions[154]. The urokinase plasminogen activator-urokinase receptor complex concentrates in the inflammatory cells in perivascular spaces, potentially facilitating cellular infiltration into the CNS. Interestingly, in the same study, tissue plasminogen activator (the most abundant plasminogen activator in MS lesions) was reduced. Finally, laminin isoforms are involved in perivascular cuffing: inflammatory cuffs only occur around endothelial basement membranes containing laminin 8 (now known as laminin 411) and laminin 10 (now known as laminin 511)[8].Of note, the differential laminin expression of laminin 511 in different parts of the vasculature defines sites of low/no laminin 511 expression only at postcapillary venules as permissive for leukocyte extravasation.

Based on this and prior reviews[108,155,156] , we have synthesized an anatomical and functional model of perivascular spaces in neuroinflammation (Figure 1C). While this review focuses on the perivascular space, for a detailed overview of BBB pathophysiology, including the adhesion molecules involved in leukocyte transmigration, please refer to other comprehensive reviews by some of the present paper's authors[112,114] and others[157,158].

6. Perivascular spaces and the central vein

The perivascular spaces surrounding postcapillary venules, which are the usual site of parenchymal lymphocyte infiltration (reviewed in[159]), play a particularly important role in neuroinflammation. Initially described by Eduard Rindfleisch[160], many authors have since reported central venules in the white matter of MS lesions in autopsy samples (reviewed in[161]). However, it has only recently become possible to image the perivenular topography of an MS lesion *in vivo* using high-resolution MRI[162] (reviewed in[163]). Far from being merely a morphological feature of MS lesions, the central vein sign is currently being implemented in clinical research studies in order to increase the specificity of MS diagnostic criteria[164]. Such lesion-centered veins are not commonly observed in MS-mimicking neuroinflammatory or non-inflammatory conditions[165].

The underlying cause of the central vein's prominence within MS lesions is still a matter of debate. Higher oxygen requirements within the lesion parenchyma (with a concomitant

increase in deoxyhemoglobin) and vessel-size alterations have been suggested[166,167]. Recent data from studies of marmoset neuroinflammatory models and MS have indicated both luminal enlargement and eccentric thickening of perivascular spaces via type I fibrillar collagen deposition[168]. However, none of these points explains why the central vein is so prominent in MS lesions in particular, despite being very rare in other neuroinflammatory diseases in which lesions also arise through perivenous inflammation. For example, the central vein sign is rare or absent in MOGAD, despite a pathology involving brain inflammation and widespread demyelination, i.e., similar pathological hallmarks as in MS[127,169]. The major difference is that one key feature of MS — the formation of large, perivascular, lymphocyte-rich, inflammatory aggregates[170] — is very rare in MOGAD, with small perivascular infiltrates around venules dominating[169]. It is possible that large inflammatory aggregates can only develop in connective tissue spaces of sufficient size, which, in the human brain, are only available in the meninges and large perivascular spaces[171].

7. Translational aspects of perivascular spaces

It is noteworthy that many of the findings described above were gleaned from rodents, especially EAE studies. This results in key caveats regarding the translation of perivascular space function and anatomy from rodents to humans. Regarding perivascular space function, although EAE is the most frequently used animal model for mimicking MS[172], its ability to model every aspect of MS has been disputed[173]. Most notably, the nature of the immune response leading to brain inflammation is different between rodent neuroinflammation and MS[173]. For example, in contrast to MS in which CD8+ T cells are suggested to play a key role during pathogenesis, EAE is mostly driven by CD4+ T cells. Antigen recognition, the mode of immune cell activation, the mechanisms of immune-mediated tissue injury, and, associated with this, the type of inflammatory reaction is supposed to be fundamentally different in inflammatory brain diseases propagated by CD4+ T-cell-mediated, CD8+ T-cell-mediated, and T-cell plus antibody-mediated inflammatory CNS diseases. In addition, no EAE model has ever convincingly described the full clinical course and pathological characteristics of progressive MS[173,174]. Thus, it has been suggested that EAE is a more accurate model for acute or relapsing disseminating encephalomyelitis, including MOGAD[174]. This is further supported by the finding that autoimmunization of humans using brain tissue (which leads to widespread MS-like demyelination) is associated with an intrathecal antibody response against MOG[175].

Regarding perivascular space anatomy, there are several similarities between rodents and humans. These include similar composition of the two cellular layers and basement membranes as well as the presence of perivascular cuffs in both MS and EAE[61]. However, there are also key differences in perivascular space anatomy between these species. Most obviously, the considerably larger size of human brains can be associated with much larger perivascular spaces, especially in periventricular white matter[171]. This should lead to substantially longer trafficking distances for the immune cells within those perivascular spaces[61], and it suggests that the pathogenetic mechanisms behind neuroinflammation (including T cell surveillance and their activation within perivascular spaces) could involve

different pathways in humans. In summary, more research is warranted to link findings from rodent studies to the human population.

8. Knowledge gaps

There are critical gaps in knowledge about perivascular space anatomy and function (Table 2). First, despite over a century of study, the exact anatomy of perivascular spaces is still debated, and the literature is hampered by inconsistent terminology. Open questions include whether there are anatomical differences between perivascular spaces at different levels along the CNS vascular tree, i.e., around veins, postcapillary venules, and arteries[5], between perivascular spaces in the centrum semiovale and the basal ganglia[176], or between cerebral and spinal cord perivascular spaces[177]. High-resolution MRI has enabled noninvasive insights into macroscopically visible EPVS, thus partially addressing these questions[26,27]. However, histological validation of these findings is largely lacking, and postmortem fixation artifacts (which can affect extracellular spaces) compound the problem. In addition, to date, MRI has been unable to identify spinal cord perivascular spaces, likely in part due to the technically limited resolution of spinal cord MRI[178]. Another cause could be anatomical differences between cerebral and spinal cord perivascular spaces[177]. Increasing spatial resolution and modern MRI techniques, including dynamic image acquisition methods, might provide pioneering insights into the anatomy of microscopic perivascular spaces and into their function in general.

MRI studies have identified several neuroinflammatory disorders with greater numbers of EPVS, mostly MS[34] and, to a lesser extent, systemic lupus erythematosus[31]. Yet, a second gap in knowledge surrounds the continuing debate over the exact role of these imaging biomarkers. Based on longitudinal MRI data, one study hypothesized that the dilation of EPVS might represent a local accumulation of immune cells prior to the emergence of a neuroinflammatory lesion[37]. However, these findings have yet to be reproduced[34]. High-resolution MRI data suggest that EPVS might, at least in part, be signs of focal *ex vacuo* brain atrophy[25]. Determining the role of EPVS in the pathology of MS and other neuroinflammatory disorders will require more neuroimaging studies, particularly longitudinal ones. Furthermore, despite its inherent difficulty, correlating EPVS with their corresponding histopathology could give key insights into their pathophysiology (Figures 2G–2I).

Although the existence of the glymphatic system remains controversial, especially in humans and particularly with respect to the fluid drainage pathways and proposed role of aquaporin-4, there is a large body of evidence supporting the existence of a convective fluid flow within perivascular spaces (though not necessarily within the parenchyma). MRI data suggest that certain CNS diseases, such as normal pressure hydrocephalus, might be associated with reduced convective flow in perivascular spaces[58]. Indeed, it has been speculated that there are similar mechanisms in Alzheimer's diseases, based on rodent studies[179] and postmortem human studies[180] (reviewed in[181]). Also, dynamic PET-tracer studies have shown altered ventricular CSF flow in MS[182]. However, a third gap in knowledge is the current uncertainty about whether fluid circulation within perivascular spaces is also altered in neuroinflammation, and, if this were the case,

whether this is a primary cause of neuroinflammatory pathology or a bystander effect. In addition, particularly in MS (with its predominant perivenular pathology), peri2 arterial and perivenous convective flow might be altered in distinct ways.

Fourth, even though several molecules have been identified as governing the priming of immune cells in perivascular spaces, this process, including the exact type of APCs, is still a matter of debate. Both dendritic cells and perivascular macrophages have been implicated as those APCs, but resident microglia may also play this role[183] by extending their processes to take part in the neurovascular unit[184]. The small dimensions of this anatomical compartment certainly impede any detailed assessment of the cells involved. However, it is clear that the involvement of perivascular spaces and their cells depends upon the type of inflammation, the pathogenic roles of different immune cells, and the nature and location of the target antigen that triggers the inflammatory reaction. Thus, more emphasis needs to be placed on disease-specific aspects of the immune reaction within the CNS compartment.

9. Limitations of the available evidence

There are several limitations in the available studies on perivascular spaces (Table 2). A first limitation is that the terms ‘perivascular’ and ‘paravascular’ are inconsistently used throughout the literature. We recommend using ‘perivascular’ to denote the compartments between the parenchymal basement membrane of the glia limitans and the vessel’s outer border[81,107] (Table 1). In addition, phrases such as “leukocytes cross the blood–brain barrier” were used in many of the studies discussed here, neglecting the presence of perivascular spaces and thus diminishing the clinical relevance of distinguishing between whether these cells had passed through the vessel walls of postcapillary venules into the perivascular space or had in addition progressed through the glia limitans into the neuropil[109]. Furthermore, inflammatory cells may remain in perivascular spaces but induce clinical disease and tissue damage by producing soluble factors. Anti-MOG antibodies in EAE and MOGAD are examples of this mechanism[169,185].

A second limitation is that many of the discussed studies assessing perivascular spaces did not properly define the exact delineations of their perivascular spaces by means of specific immunostaining and/or electron microscopy. We recommend that future studies include such immunostainings, e.g., for pan-laminin[8] (Figure 2B) or other markers labelling specific components of perivascular spaces, such as collagen type IV (for labelling the basement membranes of the perivascular space) and laminin-alpha 1, β-dystroglycan, or aquaporin-4 (for its outer boundary). In this context, it is also important that MRI detection of perivascular spaces is restricted to macroscopically visible Virchow-Robin spaces, which form only a fraction of perivascular spaces in the CNS. Thus, findings from MRI studies require validation by histopathology before results can be generalized.

Many similarities between rodent and human perivascular space anatomy exist, yet critical distinguishing features, such as size differences, warrant a more careful interpretation of the findings from preclinical studies. This limitation may reduce the relevance of rodent data for human physiology and disease[186]. A focus should therefore be put on performing more human studies, particularly using modern, noninvasive neuroimaging approaches and,

if possible, combining them with neuropathology in direct comparative investigations of postmortem material. The advent of ultra-high-resolution MRI enabling near-microscopic insights into perivascular space anatomy, and potentially even a visualization of the fluid dynamics within them (e.g., via phase-contrast imaging), might lead to the next generation of perivascular space studies. Additionally, studies in nonhuman primates, such as marmosets, might further bridge the gap between rodents and humans.

10. Conclusions

Our review corroborates the key role of perivascular spaces in neuroinflammatory pathologies such as MS but also during immune surveillance. However, despite many years of intense research on this topic, many critical gaps in knowledge remain, such as the exact anatomy of perivascular spaces, the etiopathogenesis of EPVS in neuroinflammatory diseases, and the role of fluid dynamics within perivascular spaces. Furthermore, technical limitations and uncertainties as to how findings translate from animal disease models to humans suggest the need for careful interpretation of the available evidence. An improved understanding of these anatomical compartments could lead into promising yet uncharted therapeutic territory.

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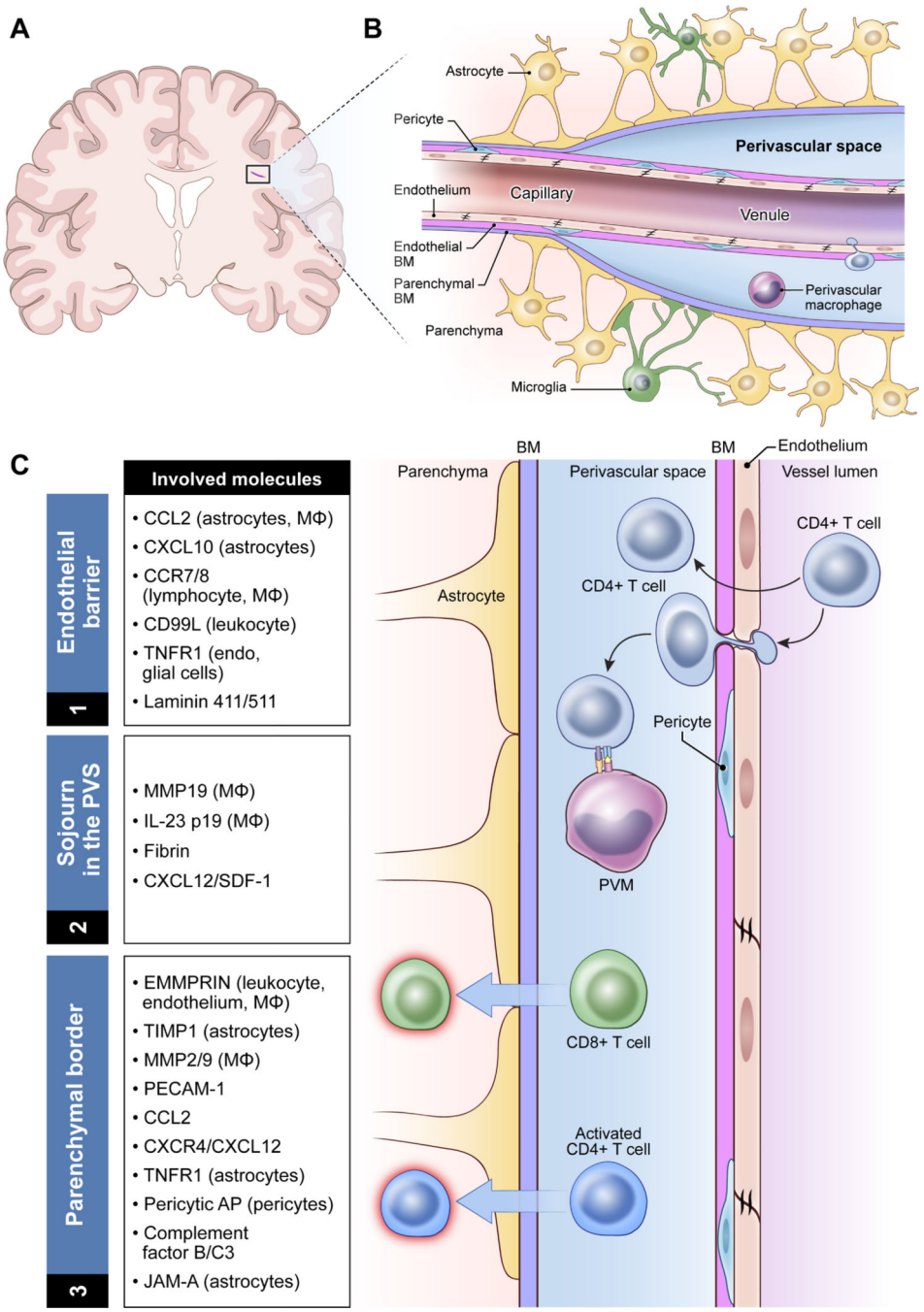

Figure 1:

Anatomy and functions of the perivascular space at the level of a postcapillary venule. A: Location of perivascular spaces within the brain. B: Anatomical model of a perivascular space at the level of the postcapillary venule. C: Functional model of immune cell trafficking within the perivascular space of a postcapillary venule, including the relevant molecules identified to date. Note that for a more comprehensive list of adhesion molecules on the endothelium, we refer to other reviews.[112–114].

Abbreviations: AP, amino peptidase; BM, basement membrane; EMMPRIN, Extracellular matrix metalloproteinase inducer; Endo, endothelial cells; IL, interleukin; JAM-A, junctional adhesion molecule-A; Leuko, leukocytes; MΦ, macrophage; MMP, matrix metalloproteinase; PECAM, platelet endothelial fundingcell adhesion molecule; PVM, perivascular macrophage; TIMP, tissue inhibitor metalloproteinase; TNFR, tumor necrosis factor receptor.

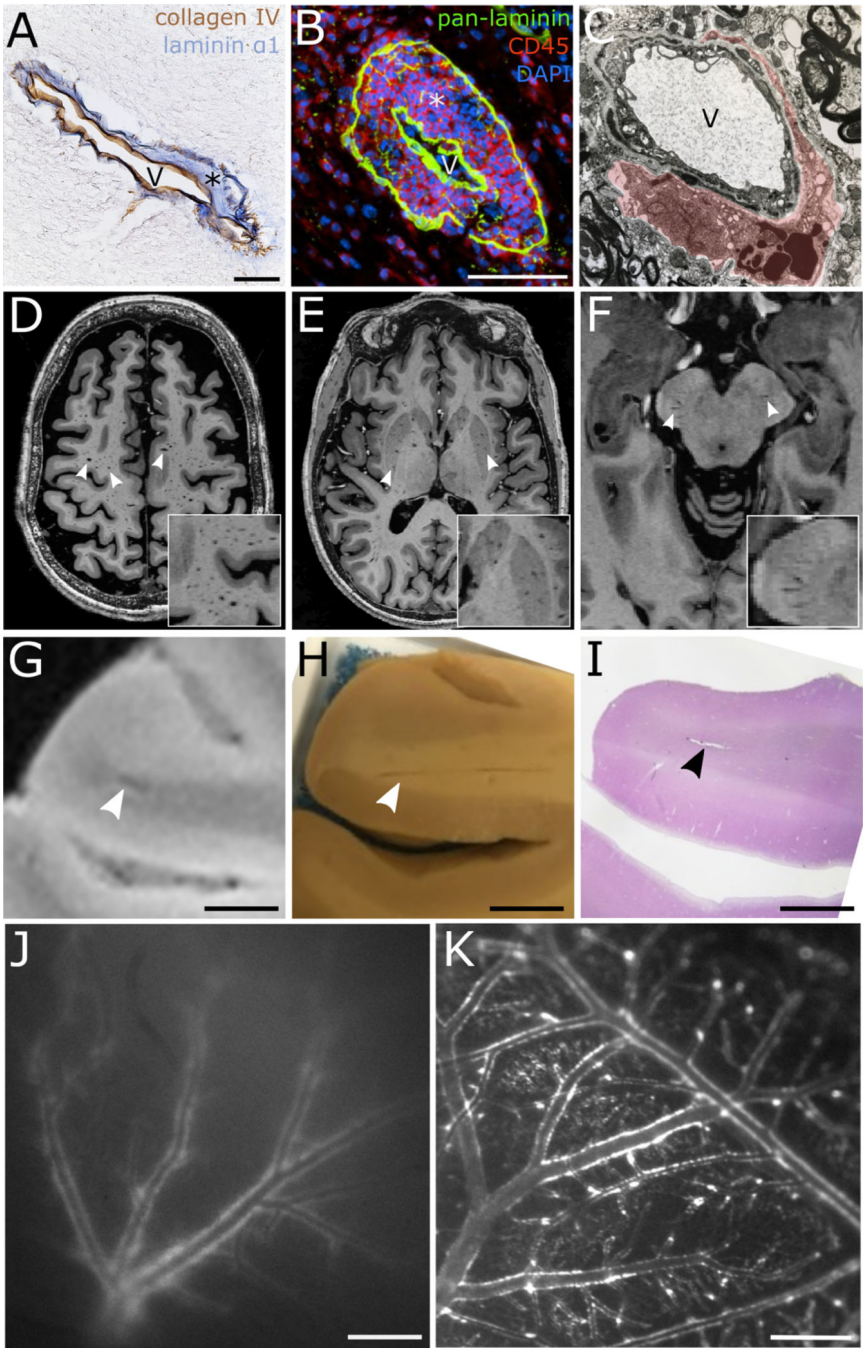

Figure 2:

Visualization of (enlarged) perivascular and ‘paravascular’ spaces.

A: Visualization of a perivascular space using double immunohistochemistry for collagen type IV (brown, vascular basement membrane) and laminin α1 (blue, parenchymal basement membrane) (*labels the perivascular space, V = vessel lumen; magnification bar: 50 μm). B: Histological visualization of a perivascular space at the level of a postcapillary venule using triple immunofluorescence staining for pan-laminin (green, labeling both the parenchymal and vascular basement membranes), leukocytes (CD45, red), and cell nuclei

(DAPI, blue). On the induction of experimental autoimmune encephalomyelitis (EAE) in mice, leukocytes accumulate in perivascular spaces before parenchymal invasion, as in this example (magnification bar: 50 μm). C: Electron microscopy image of a perivascular space and containing two macrophages in the lower portion (red shading labels the perivascular space; magnification: 15,750x). D–F: 7-tesla magnetic resonance imaging (MRI) of enlarged perivascular spaces (EPVS, T1-weighted MRI scans, white arrowheads). EPVS have signal intensities similar to cerebrospinal fluid and are commonly observed in the centrum semiovale/supratentorial white matter (D), the basal ganglia (E), and/or at the pontomesencephalic junction (F). Inset images show higher magnifications of EPVS. G–I: MRI-histology correlation of an MRI-visible Virchow-Robin space (magnification bars: 5 mm). Postmortem 7-tesla MRI depicting a juxtacortical Virchow-Robin space (G, arrowhead) with a corresponding Virchow-Robin space on gross pathology (H) and on histology (I, H&E staining). J: Tracer-filled ‘paravascular’ spaces of superficial brain blood vessels as visualized by *in vivo* near-infrared imaging through an intact mouse skull (tracer size: 40 kDa; magnification bar: 500 μm). K: *Ex vivo* distribution of tracer around arteries and veins on the surface of the cortex in the ‘paravascular’ spaces (tracer size: 40 kDa, magnification bar: 500 μm). Note that the tracer distribution becomes more prominent at the time of death[55].

Table 1:

Definition of key terms used in this review.

Term	Definition
Perivascular space	The compartment surrounding (from the Ancient Greek: *peri* – around) a brain or spinal cord blood vessel, that is, arteries, arterioles, venules, and veins, and located within the parenchyma This is not necessarily a fluid space but could also be filled with extracellular matrix.
Periarterial and perivenous space	The perivascular space surrounding an artery or vein, respectively.
'Paravascular' space	From the Ancient Greek *para* (next to), the non-concentric compartment alongside blood vessels in the subarachnoid or subpial space on the surface of brain and spinal cord, as recently visualized during intravital microscopy.
Virchow-Robin space	Large perivascular spaces that are visible macroscopically or on magnetic resonance imaging (MRI).
Glia limitans	Formed by compacted astrocyte foot processes and an overlying parenchymal basement membrane

Table 2:

Key gaps in knowledge gaps on perivascular space pathophysiology and limitations of the available literature.

Key knowledge gaps
1) Perivascular space anatomy -What is the exact anatomy of perivascular spaces? -Are there anatomical differences between periarterial, periarteriolar, perivenular (including peripostcapillary venule), and perivenous spaces?
2) Magnetic resonance imaging (MRI)-visible enlarged perivascular space (EPVS) etiopathogenesis -How do EPVS relate to arteries and/or veins? -What is the temporal evolution of EPVS and why do they become enlarged, i.e., MRI-visible? -How do EPVS relate to perivascular spaces around postcapillary venules where immune cell trafficking occurs?
3) Fluid dynamics of perivascular spaces -Is there directional fluid influx along periarterial and outflow along perivenous spaces? -Are fluid dynamics within perivascular spaces disrupted in neuroinflammatory diseases such as multiple sclerosis (MS)?
4) Neuroinflammatory priming within the perivascular space -Which cells serve as antigen-presenting cells in the perivascular space, e.g., macrophages, dendritic cells, B cells? -Which mechanisms govern differences between homeostatic immune-cell trafficking within the perivascular space and the trafficking that occurs during a neuroinflammatory outbreak?
Limitations of the available literature
1) Inconsistent terminology -“Paravascular” versus “perivascular” -Leukocytes “cross the blood-brain barrier”
2) Insufficient anatomical delineation of the perivascular space/compartment, including insufficient definition of locations within the vascular tree (artery, arteriole, venule, vein)
3) Unclear translatability of animal study findings -Caused by anatomical differences, e.g., between human and rodents